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IN-VITRO SCREENING METHODS EVALUATING THE NEUROTOXIC POTENTIAL OF PESTICIDES

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The abdominal nerve cord preparation isolated from the crayfish is far superior to the frog neuromuscular and crayfish neuromuscular preparations for evaluation of neurotoxicity of various pesticides and other environmental agents. Neurotoxicity is manifested as stimulation and/or paralysis of spontaneous discharges of the nerve cords. The techniques involved in this experiment are rather simple, and require only reasonable amounts of conventional electrophysiological equipment. Thus such toxicity evaluation can be performed with a modest amount of expense. More sophisticated and efficient data acquisition systems could be developed using basically the same techniques.

The order of potency of various insecticides in stimulating the crayfish abdominal nerve cord is as follows: allethrin = carbofuran > carbaryl > Baygon (propoxur) = p,p'-DDT = toxaphene > ferbam = monocrotophos = dursban = chlordimeform > leptophos = dichlofenthion. No neurotoxic effect is exerted by insect sex attractants and insect growth regulators including Altosid, Orfamone, Codlemone, ZR 777, cis-11 tetradecenyl acetate, and TH 6040.

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I. INTRODUCTION

Toxic effects of a variety of pesticides and environmental agents have been a matter of public concern during the past decade. Toxicity may appear in different forms in terms of the symptoms of poisoning, the speed of action, and the severity of effects. Certain pesticides and environmental agents are potent neuropoisons, some are metabolic poisons, and some others are carcinogenic compounds. In view of the increasing number of pesticides and environmental agents which come into contact with public life, it is urgently needed to develop methods whereby toxicity of such agents can be evaluated with a minimum amount of cost and time and with a maximum degree of accuracy and reliability. This contract work was aimed at developing such methods for neurotoxic pesticides and environmental agents.

Several isolated nerve and muscle preparations have been tested for their suitability for such toxicity evaluation. It was found that the abdominal nerve cord preparations isolated from the crayfish were far more suitable than other preparations examined.

II. METHODS

Crayfish Abdominal Nerve Cord

Sources of animal.

The crayfish Procambarus clarkii were purchased from several animal supply houses.

Maintenance of animal.

The crayfish were kept alive in kitchen plastic trays (about 12 x 9 x 5 inches) at a room temperature of 22°C. Some of 6 to 10 crayfish were put in a tray containing tap water in a depth of approximately 2 inches. No attempt was made to feed them. Water was changed every other day. Some of the crayfish died within a week after delivery. However, those which survived the first week usually lived

for more than several weeks, and no difficulty was encountered in maintaining constant supply to the laboratory work.

Isolation of the abdominal nerve cord.

All legs and claws were severed from their origin, and the crayfish was mounted with the ventral side up on a wooden board (8 x 6 x 0.5 inches) by means of small nails. The ventral cuticle of the abdomen was removed by means of scissors. Care was taken not to damage the abdominal nerve cord which was located immediately beneath the ventral cuticle. Then both ends of the abdominal nerve cord were tied with silk threads. The threads were attached to the outside of the first and last abdominal ganglia so as not to damage these two ganglia. All branches originating from each ganglion were cut, and the entire abdominal nerve cord was isolated without damaging any ganglion. van Harreveld solution was often dropped onto the open abdomen to prevent the nerve cord from drying up.

Nerve chamber.

Several types of nerve chambers were compared for their suitability for the present purpose. The type which was finally adopted is diagrammatically shown in Figure 1. It was made of plexiglass. The structure is so simple that it takes only a few hours to make one.

The nerve chamber consists of three compartments. The central compartment is equipped with a pair of wire recording electrodes. Hypodermic needles (gauge 24) perfectly serve for this purpose. The central compartment is also equipped with an inlet and an outlet for bathing medium. Each of the side compartments is equipped with a hypodermic needle (gauge 24). This needle serves to tie off the thread attached to the nerve preparation.

The isolated abdominal nerve cord was mounted on the electrodes in the central compartment of the chamber, and the compartment was filled with van Harreveld

solution. The entire chamber was covered by a glass plate to prevent the nerve from drying up. When recording was to be made, the solution was drained by suction through the outlet of the central compartment. The nerve cord was now hung in air on the electrodes. This increased the external electrical resistance between the two recording electrodes thereby increasing the amplitude of the recorded action potentials.

Electronics recording equipment.

The isolated abdominal cord generated action potentials spontaneously. They originated from the ganglia. The spontaneous discharges recorded as described above were in the order of millivolts in amplitude and about 100 per second in frequency. The amplitude and frequency were maintained fairly constant for 3 hours or longer.

Spontaneous discharges from the isolated nerve cord were amplified by a RC coupled amplifier and displayed on a cathode ray oscilloscope. An audio monitor was often used for long-term experiments, and was very helpful in detecting changes in frequency of discharges. Any oscilloscope equipped with an amplifier could be used for this purpose with a gain of 0.2 - 1 mV/cm on the face of the scope.

Spontaneous discharges displayed on an oscilloscope were photographed on a film using the Nihon-Kohden Oscilloscope Camera. More convenient way to acquire data was to use an electronic counter. The Tektronix DC-503 Electronic Counter proved quite satisfactory for this purpose. The recorded action potentials were amplified and fed into the counter to directly read the frequency of discharges.

Physiological saline solution.

van Harreveld solution (van Harreveld, 1936) was used as the physiological saline solution for the crayfish nerve cord. Tris buffer was substituted for bicarbonate buffer in the original solution. The solution used contained

207.3 mM NaCl, 5.4 mM KCl, 13.0 mM $CaCl_2.2H_2O$, 2.6 mM $MgCl_2.6H_2O$, 1.9 mM Trizma HCl, and 0.4 mM Trizman Base with a final pH adjusted to 7.55.

Frog Neuromuscular Junction.

Sources and maintenance of animal.

The frogs Rana pipiens were purchased from various animal houses, and were kept in containers with dripping tap water. For the present purpose, small frogs were preferred to large frogs, because the equilibrium of the muscle with a test compound was reached sooner with small muscles than with large muscles

Isolation of the sciatic nerve-sartorius muscle preparation.

The frog was decapitated, pithed with a fine needle, and skinned. The sartorius muscle was isolated with a short length of the sciatic nerve attached. Both ends of the muscle were tied with silk threads.

Muscle chamber.

The muscle chamber used for the frog sartorius was a glass cyclinder with 25 mm in diameter and 80 mm in height. A rubber stopper was fitted into the bottom of the cylinder. A metal hook attached to the rubber stopper served to fix one of the threads that tied the muscle. The other thread that tied the muscle was connected with a force displacement transducer (Grass model FT03C). Ringer's solution was introduced to the bottom of the chamber through a glass capillary, while it was sucked up from the surface of the fluid through another capillary. A pair of silver-silver chloride electrodes were placed on both sides of the muscle for direct (muscle) stimulation. The nerve was stimulated via a suction electrode which was made of a glass capillary with a spiral silver-silver chloride wire outside and another spiral silver-silver chloride wire inside.

Stimulation and recording.

The nerve and the muscle were stimulated alternately at a frequency of 0.2HZ. Supramaximal stimuli were used. Isometric contractions of the muscle were

recorded on a Grass Polygraph (model 5D) via a transducer.

Physiological saline solution.

Ringer's solution was used to bath the frog nerve-muscle preparation. I contained 115 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2.2H_2O$, 2.15 mM $Na_2HPO_4.7H_2O$, and 0.85 mM $NaH_2PO_4.H_2O$ with a final pH adjusted to 7.2.

Crayfish Neuromuscular Junction.

Stimulation and recording.

A claw was severed from the crayfish. The tip of the one side of the claw was chopped off and inserted into a plastic tubing which was in turn connected to an Ag-AgCl wire via van Harreveld solution. Another Ag-AgCl wire was placed near the proximal cut end of the claw. These two wires served for direct muscle stimulation. A suction electrode similar to that used for the frog preparation was used to stimulate the nerve. Isometric muscle contractions were recorded on a Grass Polygraph via a transducer as in the case of the frog muscle.

Test Compounds

Test compounds were dissolved in pure ethanol in a concentration of 1×10^{-1}M to 1×10^{-2}M to make up stock solutions. Immediately before use, the stock solution was diluted with physiological saline solution to give a desired final concentration of the compound. Control experiments were performed to see the effect of ethanol itself.

Temperature

All experiments were conducted at a room temperature of 22°C.

III. RESULTS

Crayfish Abdominal Nerve Cord

Under normal conditions, the abdominal nerve cord elicited spontaneous discharges at a frequency of 50-100/sec. The amplitude of action potentials was of the order of millivolts. The action of a test compound may be manifested by an increase or a decrease in the frequency of discharges. A biphasic effect could also be observed, with a usual pattern of a transient increase followed by a decrease in frequency. These effects were produced within 30-60 min after introduction of a test compound in a manner dependent upon the concentration. In the absence of test compounds, spontaneous discharges were maintained at a constant level for as long as 3 hours.

In addition to pesticides and other environmental chemicals provided by the EPA for test, three insecticides were tested as models, including p,p'-DDT, allethrin and toxaphene.

Organophosphorus and carbamate insecticides.

The effects of allethrin, p,p'-DDT, toxaphene, and organophosphorus and carbamate insecticides on the spontaneous discharges are summarized in Table 1. Detials of the effect of each compound are described below.

Ethanol control.

Since all test compounds were dissolved in ethanol to make up stock solutions, the effect of ethanol itself was examined first. Figure 2 illustrates the frequency of spontaneous discharges of the abdominal nerve cord before and during application of 1% ethanol (v/v) and after washing with ethanol-free van Harreveld solution. Ethanol caused no appreciable effect on the frequency of discharges over a period of 30 minutes. Thus it can be concluded that ethanol may be used as a solvent as long as itsfinal concentration does not exceed 1%.

p,p'-DDT.

p,p'-DDT has been known to stimulate the abdominal nerve cord of the cockroach to increase discharge frequency (see Narahashi, 1971, for references). DDT was also effective in stimulating the crayfish abdominal nerve cord as shown in Figure 3. The effect was moderate with 1×10^{-5}M DDT, and was not reversed after washing with DDT-free solution. At a concentration of 1×10^{-4}M, the increase in discharge frequency was more pronounced, the level after a 30-min application attaining approximately 300% of that of the control. The effect was not reversed but rather augmented after washing with DDT-free solution.

Toxaphene.

Toxaphene was effective in increasing the frequency of spontaneous discharges (Figure 4). The effects were almost the same with 1×10^{-5}M and 1×10^{-4}M, toxaphene, and the frequency was elevated to approximately 200% of that of the control.

Allethrin.

Allethrin and other pyrethroids have been known to stimulate and paralyze the abdominal nerve cord preparations of the cockroach and crayfish (Narahashi,1971; Camougis and Davis, 1971; Burt and Goodchild, 1971a,b; Camougis, 1973). In the present experiments, allethrin was found to be most potent among the compounds tested to stimulate and paralyze the crayfish abdominal nerve cord (Figure 5A).

The results of typical experiments are illustrated in Figures 5B to 5E. Even at a very low concentration of 1×10^{-8}M, allethrin caused a sizable increase in the frequency of discharges. Each dot in Figure 5 and in subsequent figures for other chemicals represents single measurement of the frequency for a period of either 0.2 sec or 1 sec, and the measurements were repeated as are shown by several dots. Large scatters of dots at each time during the latter half of application of 1×10^{-8}M allethrin (Figure 5B) indicate periodic bursts of high frequency discharges. Similar effects were obtained with 1×10^{-7}M

allethrin (Figure 5C).

At a concentration of 1×10^{-6}M, the effect of allethrin was represented by a transient increase in frequency followed by a decrease and an eventual paralysis (Figure 5D). The stimulation occurred very quickly in 5 min after application. At a concentration of 1×10^{-5}M, a transient increase in discharge frequency was quickly followed by a complete paralysis (Figure 5E). Recovery after washing with allethrin-free solution was very poor or absent regardless of the concentration.

Carbofuran.

Carbofuran was the only test compound that exhibited a potent stimulating action comparable to that of allethrin. At a concentration of 1×10^{-8}M, the frequency of discharges was steadily increased attaining a maximum in 15 min (Figure 6A,B). After that, the frequency tended to decrease over a period of 45 min. Essentially the same effect was observed with 1×10^{-6}M carbofuran (Figure 6C). At a concentration of 1×10^{-5}M, however, a depressive action was observed after a transient increase in frequency (Figure 6D). At a concentration of 1×10^{-4}M, no clear-cut stimulation resulted, and the nerve cord was paralyzed completely (Figure 6E). Recovery after washing with solution free of carbofuran was incomplete (Figure 6E).

Carbaryl.

Carbaryl was not as potent as carbofuran or allethrin, but slightly stimulated the nerve cord at a concentration of 1×10^{-6}M (Figure 7A). Although the average frequency was not substantially increased, burst of high frequency discharges occurred toward the end of a 60-min application of carbaryl as is illustrated by a dot at a high frequency level. At a concentration of 1×10^{-5}M, the stimulating effect was much more pronounced and was followed by a tendency toward paralysis (Figure 7B). At a concentration of 1×10^{-4}M, an exceptionally

large transient increase in discharge frequency was quickly followed by a complete paralysis (Figure 7C). Partial recovery occurred after washing with carbaryl-free solution.

Leptophos.

The effect of leptophos on the abdominal nerve cord was not particularly striking. At a concentration of 1×10^{-5}M, the frequency of discharges tended to increase transiently (Figure 8A). At a concentration of 1×10^{-4}M, a small transient increase was followed by a gradual decline of frequency (Figure 8B).

Baygon (Propoxur).

Baygon was moderately effective in stimulating and paralyzing the nerve cord. It had no effect on discharges at a concentration of 1×10^{-7}M. At a concentration of 1×10^{-6}M, a transient and slight increase in discharge frequency occurred without sign of paralysis (Figure 9A). At a concentration of 1×10^{-5}M, bursts of discharges became evident as shown by scattered dots in Figure 9B. A large transient increase in frequency followed by a paralysis occurred at a concentration of 1×10^{-4}M (Figures 9C).

Ferbam.

The effect of ferbam at a concentration of 1×10^{-6}M or 1×10^{-5}M was not obvious (Figure 10A). At a concentration of 1×10^{-4}M, bursts of discharges were produced as shown by scattered dots in Figure 10B.

Dichlofenthion.

Dichlofenthion had no appreciable effect on discharge frequency at a concentration of 1×10^{-5}M. It caused some bursts of discharges at a concentration of 1×10^{-4}M, but the effect was never striking (Figure 11).

Monocrotophos.

Monocrotophos had no effect on discharges at a concentration of 1×10^{-6}M. It caused a small and transient increase in frequency at 1×10^{-5}M, but the effect

was not remarkable (Figure 12A). Only at a concentration of 1×10^{-4}M, the stimulating effect became obvious but was never striking (Figure 12B). There was no sign of paralysis at any of these concentrations.

Dursban.

The effect of dursban at a concentration of 1×10^{-6}M or 1×10^{-5}M was almost negligible (Figure 13A). At a concentration of 1×10^{-4}M, however, dursban caused an increase in discharge frequency which was maintained over a period of 50 min (Figure 13B). Bursts of discharges also appeared.

Sex attractants, growth regulators and chlordimeform.

The effects of several insect sex attractants, insect growth regulators, and chlordimeform on the spontaneous discharges of the crayfish abdominal cord are summarized in Table 2. Examples of experiments with the insect growth regulator Altosid and the codling moth sex attractant Codlemone are illustrated in Figures 14 and 15, respectively.

It is clearly seen that all of the insect growth regulators and sex attractants tested (Altosid, Orfamone, ZR777, cis-11 tetradecenyl acetate, TH 6040, and Codlemone) have no effect on the spontaneous discharges of the crayfish abdominal nerve cord even at a very high concentration of 1×10^{-4} g/ml. The insecticide chlordimeform had a moderate stimulating effect at a concentration of 1×10^{-4} g/ml.

Frog Neuromuscular Junction.

The sciatic nerve-sartorius muscle preparation of the frog has been examined for its usefulness for evaluating the neurotoxicity of various pesticides. Since the neuromuscular junction of this preparation is nicotinic in nature, control experiments were performed with a typical nicotinic blocking agent and a typical anticholinesterase agent. d-Tubocurarine and edrophonium were used for this purpose.

However, it was found that this preparation was not as sensitive as the crayfish abdominal nerve cord to various compounds. Therefore, only several test compounds were selected for the test. The results are summarized in Table 3.

d-Tubocurarine.

d-Tubocurarine blocked neuromuscular transmission completely at a concentration of 5×10^{-6}M. The contraction induced by muscle stimulation was suppressed only slightly. The effects were slowly reversed after washing the preparation with drug-free Ringer. Figure 16 illustrates an example of such an experiment. The observed strong neuromuscular blocking action of d-tubocurarine is to be expected, and the results indicate that the method used here is satisfactory for examining the neuromuscular blocking action of nicotinic agents.

Edrophonium.

The next control experiments were done using edrophonium which is known to inhibit cholinesterases reversibly. A fairly high concentration was required for edrophonium to block neuromuscular transmission, 1×10^{-4} M having virtually no effect. Neuromuscular transmission was effectively blocked at a concentration of 1×10^{-3} M (Table 3, Figure 17). No marked potentiation or prolongation of muscle contraction was observed at any concentrations tested. The results indicate that although this preparation responds to the neuromuscular blocking action of an anticholinesterase in a predicted manner, it may not be suitable to check the neuromuscular stimulating action of an anticholinesterase.

Allethrin.

Allethrin was also examined for the purpose of comparison. The muscle contraction evoked by nerve stimulation was augmented somewhat at low concentrations (10^{-6} M - 10^{-5}M), whereas it was suppressed at higher concentrations (1-5 $\times 10^{-4}$M) (Figure 18, Table 3). The contraction induced by direct stimulation was also

decreased to some extent at high concentrations. Thus the frog neuromuscular preparation responds to the action of allethrin, but the sensitivity falls short of that of the crayfish nerve cord preparation, the difference being of the order of 1000.

Carbofuran.

As described in a previous section, carbofuran was among the most potent insecticides in stimulating and paralyzing the crayfish abdominal nerve cord (Table 1). Therefore, carbofuran was tested on the neuromuscular preparation of the frog. As is shown in Figure 19, the response to indirect nerve stimulation was suppressed more effectively than that to direct muscle stimulation by 2.5×10^{-4}M carbofuran. Data with various concentrations of carbofuran are given in Table 3. When compared with the crayfish abdominal nerve cord, the frog neuromuscular preparation is much less sensitive to carbofuran. The former preparation was affected at a concentration of 1×10^{-6}M, whereas the latter was affected only when the concentration was increased to 1×10^{-5}M, the difference in sensitivity being 1000-fold.

Leptophos.

At concentrations up to 2.5×10^{-4} M leptophos had virtually no effect on the muscle contraction as evoked by either indirect or direct stimulation (Figure 20, Table 3).

Dichlofenthion.

Dichlofenthion (1×10^{-3} M) had little or no effect on the muscle contraction evoked by either direct or indirect stimulation (Figure 21) except one case where the nerve evoked contraction was drastically reduced (Table 3).

Crayfish Neuromuscular Junction.

The nerve-muscle preparation of the crayfish has been examined for its usefulness for evaluating the neurotoxicity of various pesticides. Since the neuromuscular junction of this preparation is not nicotinic but glutaminergic in nature, different results are expected from those with the frog neuromuscular junction. It was found that this preparation was particularly insensitive to organophosphate and carbamate insecticides. It was, however, very sensitive to allethrin.

The results are summarized in Table 4.

Allethrin.

Allethrin stimulated and paralyzed the crayfish neuromuscular preparation very effectively (Figure 22). Even at a concentration of 1×10^{-8} M, it augmented the muscle contractions produced by either nerve or muscule stimulation (Table 4).

Edrophonium.

This anticholinesterase had little or no effect on the crayfish neuromuscular junction at 1×10^{-4} M. At a higher concentration of 1×10^{-3} M, it suppressed the muscle response evoked by nerve stimulation, but had no effect on the response produced by direct stimulation (Figure 23, Table 4).

Carbofuran.

Despite a potent action on the crayfish abdominal nerve cord, carbofuran (1×10^{-4} M) had no effect on the muscle responses evoked by either nerve or muscle stimulation (Figure 24, Table 4).

Dichlofenthion.

At a concentration of 1×10^{-5}M, dichlofenthion had no effect on the muscle response evoked by either direct or indirect stimulation (Table 4). At a concentration of 1×10^{-4} M, an increase in response was obtained in three cases,

whereas a decrease in response was obtained in three other cases (Figure 25, Table 4).

Leptophos.

At concentrations up to 2.5×10^{-5}M, leptophos had no effect on the muscle response evoked by nerve stimulation (Figure 26, Table 4).

IV. DISCUSSION

It is clearly shown that the crayfish abdominal nerve cord preparation is much more sensitive than the frog neuromuscular preparation to various neurotoxic agents. Differences in sensitivity are particularly evident for allethrin and carbofuran. The nerve cord is 1,000 times more sensitive than the frog muscle to either of these chemicals. Although the crayfish neuromuscular preparation is as sensitive to allethrin as the crayfish abdominal nerve cord, it is not sensitive to organophosphate and carbamate insecticides. Thus, in terms of sensitivity to various pesticides, the abdominal nerve cord of the crayfish is ranked top among the preparations examined.

The crayfish abdominal nerve cord is easiest to handle among the preparations used. The nerve cord is stable over a 3-hour period under normal conditions, and the effects of various drugs are quite reproducible. Data are obtained in the form of the frequency per unit time on a counter which is easy to operate. Though not tried in this study, it is possible to convert the digital data into an analog form which is in turn registered on a strip chart recorder. Thus data are read in a digital or analog form, depending on the availability of equipment and on the degree of need to quantitative values.

With appropriate investment, it is also possible to handle several preparations at a time to speed up the analysis. For example, with 4 channels of recording

and data acquisition system, 4 experiments can be made simultaneously. Each channel is composed of a preamplifier, a counter, a digital-to-analog converter, and a strip chart recorder. It is also possible to feed the data to a computer for analysis.

The frog neuromuscular preparation is also easy to handle and data are reproducible. However, it generally takes a longer time for the effect of a drug to attain a steady state than with the crayfish abdominal nerve cord preparation. The crayfish neuromuscular preparation is more difficult to handle, because muscle response fluctuates more widely than that of the frog muscle.

The crayfish is inexpensive and can be obtained all the year round. It is easy to keep in the laboratory for a long time without special equipment. Thus the crayfish is a highly suitable material in this respect also.

In view of these considerations, the abdominal nerve cord preparation from the crayfish is clearly the most suitable material to evaluate neurotoxicity of various pesticides and other environmental agents with reasonably simple equipment and moderate costs.

V. SUMMARY AND CONCLUSION

The abdominal nerve cord preparation isolated from the crayfish is far superior to the frog neuromuscular and crayfish neuromuscular preparations for evaluation of neurotoxicity of various pesticides and other environmental agents. Neurotoxicity is manifested as stimulation and/or paralysis of spontaneous discharges of the nerve cords. The techniques involved in this experiment are rather simple, and require only reasonable amounts of conventional electrophysiological equipment. Thus such toxicity evaluation can be performed with a modest amount of expense. More sophisticated and efficient data acquisition systems could be developed using

basically the same techniques.

The order of potency of various insecticides in stimulating the crayfish abdominal nerve cord is as follows: allethrin ≈ carbofuran > carbaryl > Baygon (propoxur) ≈ p,p'-DDT ≈ toxaphene > ferbam ≈ monocrotophos ≈ dursban ≈ chlordimeform > leptophos ≈ dichlofenthion. No neurotoxic effect is exerted by insect sex attractants and insect growth regulators including Altosid, Orfamone, Codlemone, ZR 777, cis-11 tetradecenyl acetate, and TH 6040.

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TABLE 1

SUMMARY OF THE EFFECTS OF ORGANOPHOSPHORUS AND CARBAMATE INSECTICIDES ON THE FREQUENCY OF SPONTANEOUS DISCHARGES IN THE ABDOMINAL NERVE CORD OF THE CRAYFISH

Compound	Concentration (M)				
	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}
Allethrin	+	+	++,-	++,-	
p,p'-DDT			+	+	
Toxaphene			+	+	
Carbofuran	+	+	+	+	+,-
Carbaryl		0	+	++	++,-
Leptophos			0	0	0
Baygon (Propoxur)		0	0	+	++,-
Ferbam			0	0	+
Dichlofenthion				0	0
Monocrotophos			0	0	+
Dursban			0	0	+

\+ Increase in discharge frequency.

++ Drastic increase in discharge frequency.

\- Drastic decrease in discharge frequency.

0 No change in discharge frequency.

$^{+,-}_{++,-}$ } Increase followed by decrease.

TABLE 2

SUMMARY OF THE EFFECTS OF SEX ATTRACTANTS, GROWTH REGULATORS AND CHLORDIMEFORM ON THE FREQUENCY OF SPONTANEOUS DISCHARGES IN THE ABDOMINAL NERVE CORD OF THE CRAYFISH

Compound	Concentration (g/ml) 10^{-7}	10^{-6}	10^{-5}	10^{-4}
Altosid	0	0	0	0
Orfamone	0	0	0	0
Codlemone	0	0	0	0
ZR 777	0	0	0	0
Cis-11 tetradecenyl acetate	0	0	0	0
Chlordimeform	0	0	0	+
TH 6040	0	0	0	0

\+ Increase in discharge frequency.

0 No charge in discharge frequency.

EFFECTS OF PESTICIDES AND OTHER BLOCKING AGENTS ON THE FROG MUSCLE CONTRACTION EVOKED BY DIRECT AND INDIRECT STIMULATIONS

Data are given in percentage of decrease (-) or increase (+) in contraction. Two numbers for a given concentration indicate the initial and later changes. N, nerve stimulation. M, muscle stimulation.

Compound	Concentration (M)								
	10^{-6}	5×10^{-6}	10^{-5}	5×10^{-5}	10^{-4}	2.5×10^{-4}	5×10^{-4}	10^{-3}	2×10^{-3}
d-Tubocurarine									
12-16-74A N		-97							
M		0							
12-16-74B N		-100							
M		-32							
12-26-74B N		-97							
M		-29							
12-23-74B N		-100							
M		-40							
Edrophonium									
12-24-74A N					0			-80	
M					0			-42	
12-24-74B N					0			-75	
M					0			-18	
12-23-74B N					-17				
M					0				
01-10-75A N							-22	-63	
M							-22	-47	
01-10-75B N							-70		
M							-50		
Carbofuran									
12-23-74B N			-25		-24			-100	
M			-23		-22			-34	
12-27-74A N							+15,-27		
M							0		
12-18-74A N			0		-21			-79	
M								0	
12-26-74A N					-12			-85	
M					[illegible]			-71	
01-09-75A N						-70			
M						-60			
01-08-75A N						-55			
M						-29			

TABLE 3 (Continued)

Compound		Concentration (M)								
		10^{-6}	5×10^{-6}	10^{-5}	5×10^{-5}	10^{-4}	2.5×10^{-4}	5×10^{-4}	10^{-3}	2×10^{-3}
Leptophos										
12-17-74A	N	0		0		0				
	M									
12-24-74B	N					0	+4			
	M					0	-8			
12-24-74A	N	0		0		0	0			
	M	0		0		0	0			
Dichlofenthion										
12-26-74B	N								-5	
	M								+3,-30	
12-26-74A	N					0			+9	+0
	M					0			+7	-14
01-09-75B	N							0	0	
	M							0	0	
12-24-74B	N					0			-90	
	M					0			-43	
12-16-75B	N					0				
	M									
Allethrin										
12-23-74A	N			+33		-36		-77		
	M			0		-14		-22		
01-09-75B	N							+7,-36		
	M							-37		
01-10-75C	N							-64		
	M							-46		
12-16-75B	N	+16		0		0				
	M									

TABLE 4

EFFECTS OF PESTICIDES AND EDROPHONIUM ON THE CRAYFISH MUSCLE CONTRACTION EVOKED BY NERVE AND MUSCLE STIMULATIONS

Compound		Concentration (M)						
		10^{-8}	10^{-7}	10^{-6}	10^{-5}	2.5×10^{-5}	10^{-4}	10^{-3}
Allethrin	N	+	+,-	+,-	+,-			
	M	+	+,-	+,-	+,-			
Edrophonium	N						0	-
	M							0
Carbofuran	N						0	
	M						0	
Dichlofenthion	N				0		+ or -*	
	M				0		+ or -*	
Leptophos	N			0		0		
	M							

N Nerve stimulation.

M Muscle stimulation.

\+ Increase in muscle contraction.

\- Decrease in muscle contraction.

+,- Increase followed by decrease.

* Increase in 3 cases and decrease in other 3 cases.

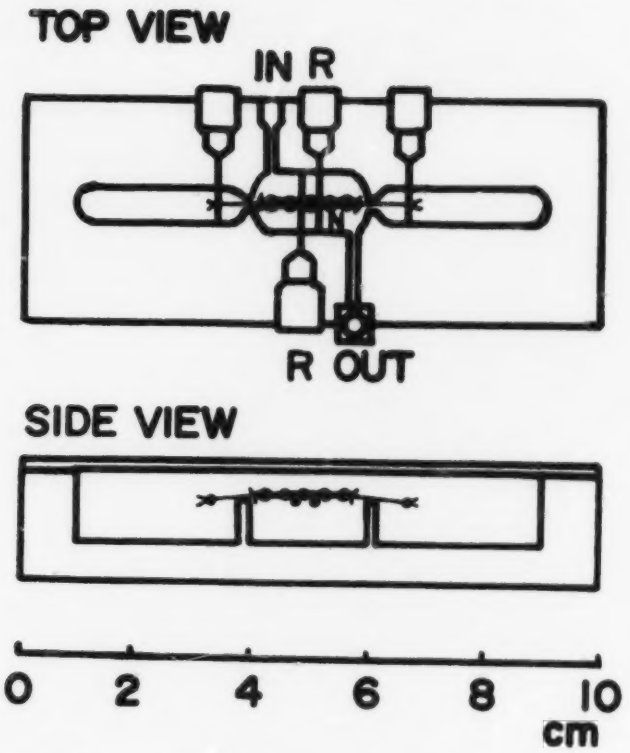

Figure 1. Diagrammatic sketch of the chamber used for the crayfish abdominal nerve cord. IN, inlet of bathing medium; OUT, outlet of bathing medium; R, recording electrode; N, nerve.

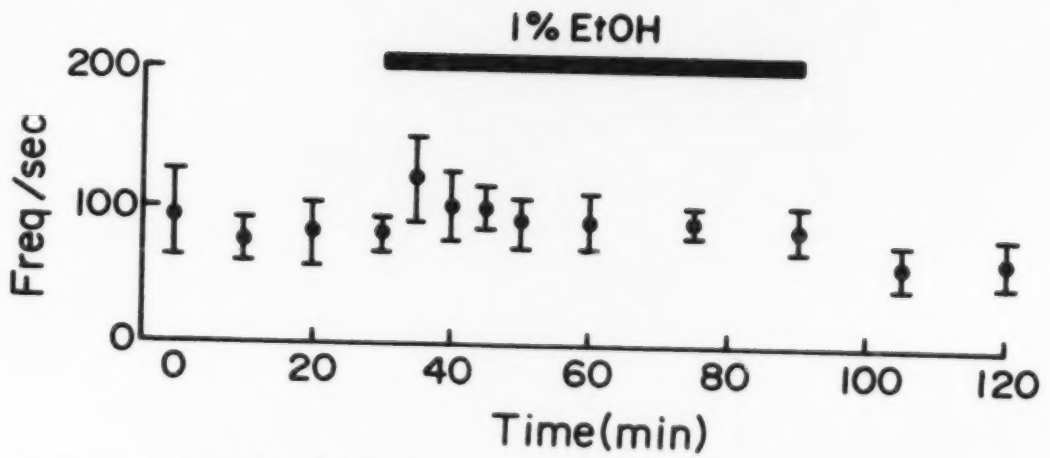

Figure 2. Effects of 1% ethanol (v/v) on the frequency of spontaneous discharges of the crayfish abdominal cord. Each point represents the mean ± standard error (4 experiments).

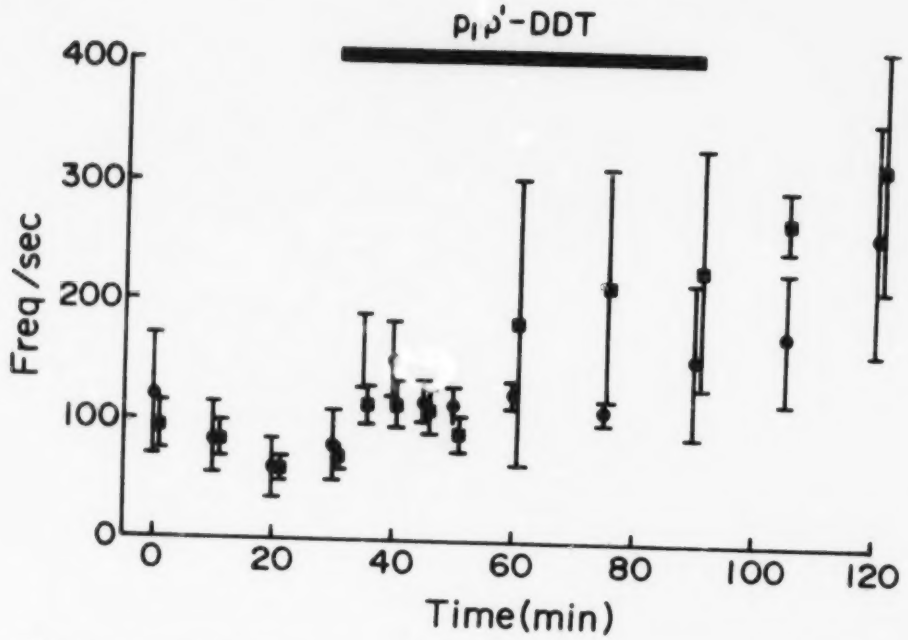

Figure 3. Effects of 1×10^{-5}M (circles) and 1×10^{-4}M (squares) p,p'-DDT on the frequency of spontaneous discharges of the crayfish abdominal cord. Each point represents the mean $\pm$ standard error (4 experiments).

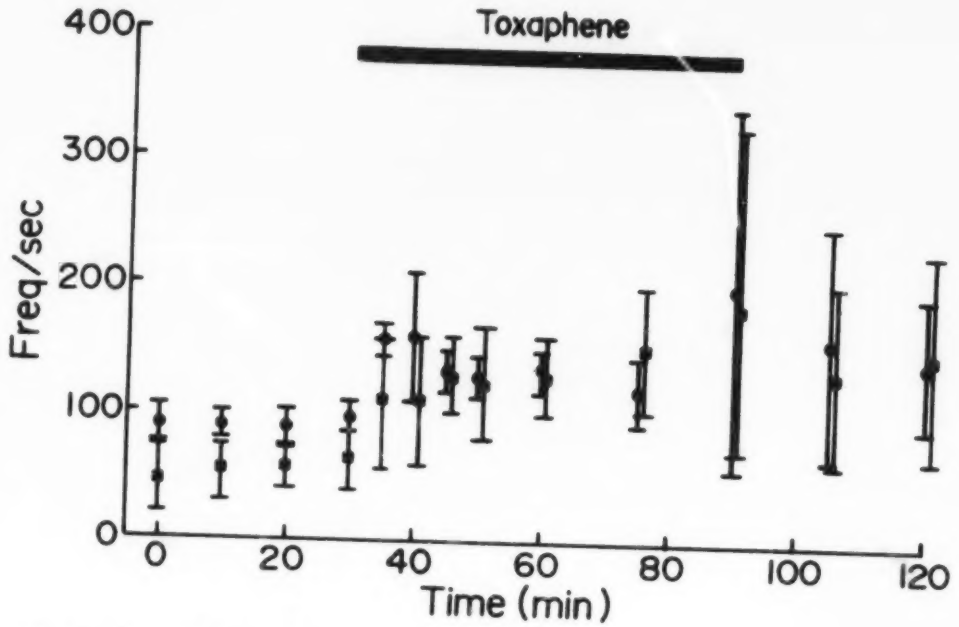

Figure 4. Effects of 1×10^{-5}M (circles) and 1×10^{-4}M (squares) toxaphene on the frequency of spontaneous discharges of the crayfish abdominal cord. Each point represents the mean $\pm$ standard error (4 experiments).

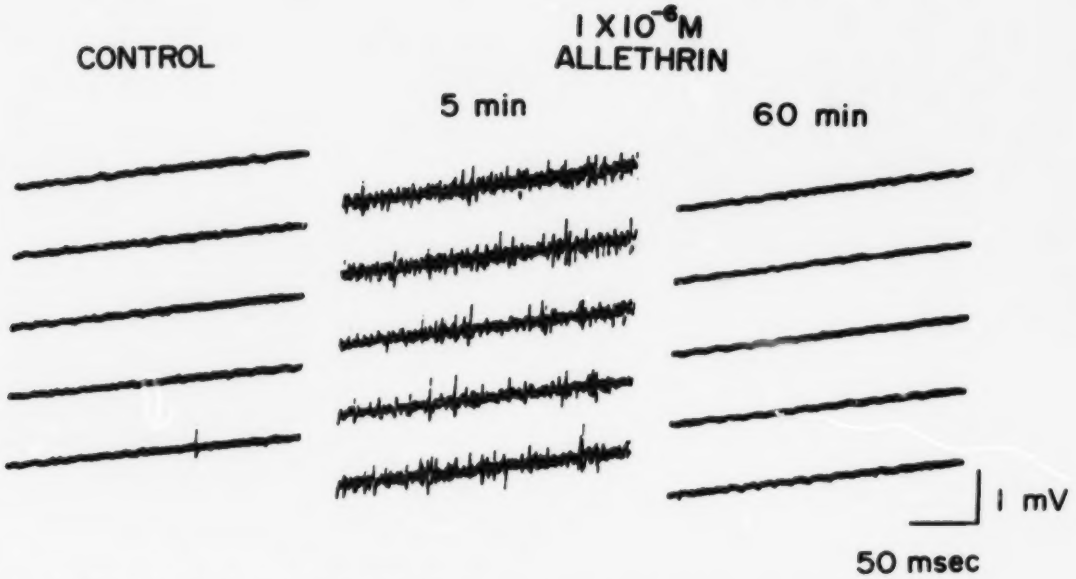

Figure 5A. Effects of allethrin on the frequency of spontaneous discharges of the crayfish abdominal nerve cord. Each point represents a measurement during a period of 0.2 or 1 sec.

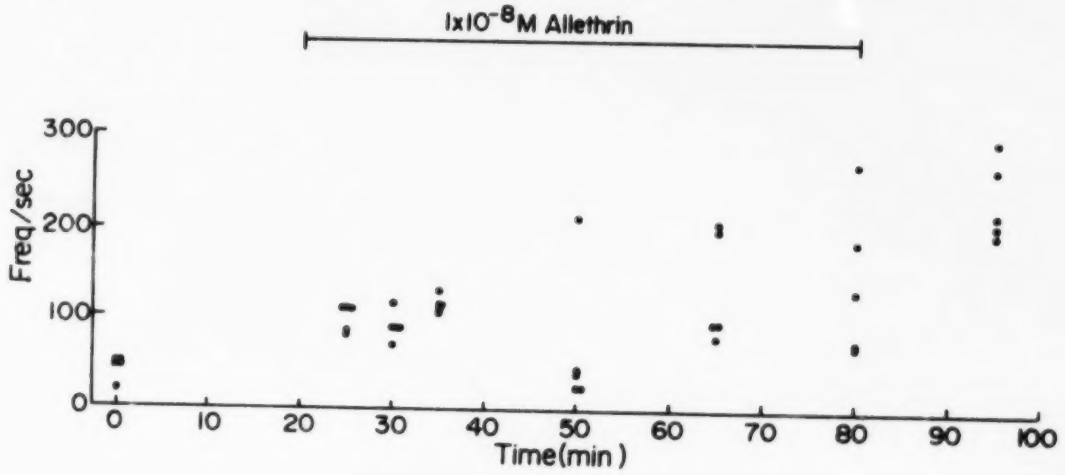

Figure 5B. Effects of allethrin on the frequency of spontaneous discharges of the crayfish abdominal nerve cord. Each point represents a measurement during a period of 0.2 or 1 sec.

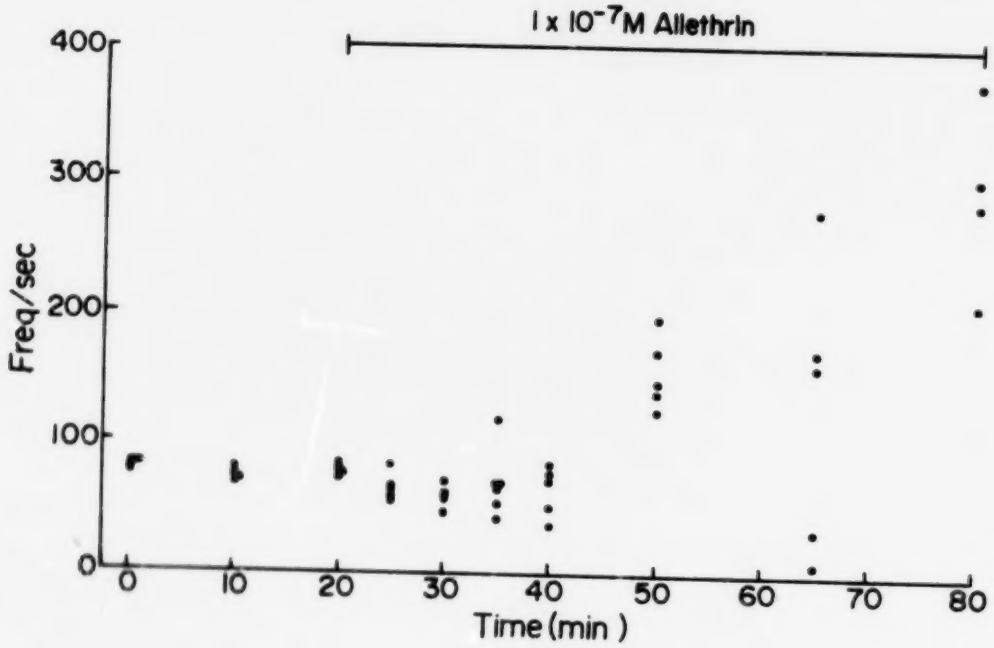

Figure 5C. Effects of allethrin on the frequency of spontaneous discharges of the crayfish abdominal nerve cord. Each point represents a measurement during a period of 0.2 or 1 sec.

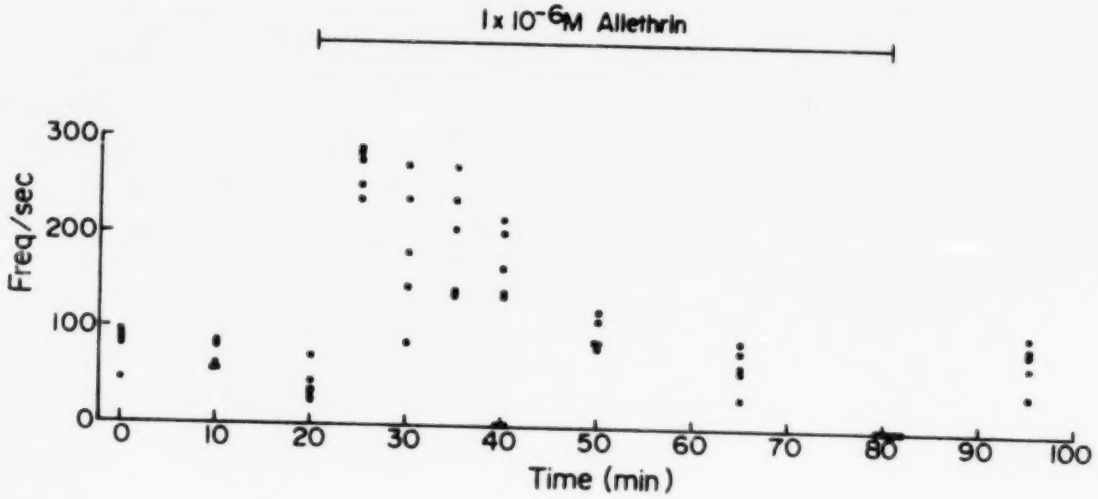

Figure 5D. Effects of allethrin on the frequency of spontaneous discharges of the crayfish abdominal nerve cord. Each point represents a measurement during a period of 0.2 or 1 sec.

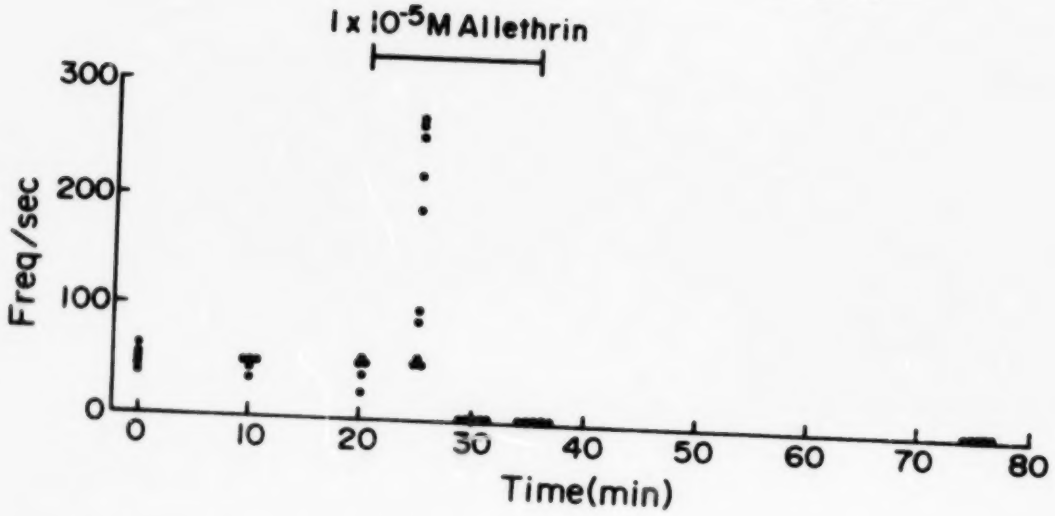

Figure 5E. Effects of allethrin on the frequency of spontaneous discharges of the crayfish abdominal nerve cord. Each point represents a measurement during a period of 0.2 or 1 sec.

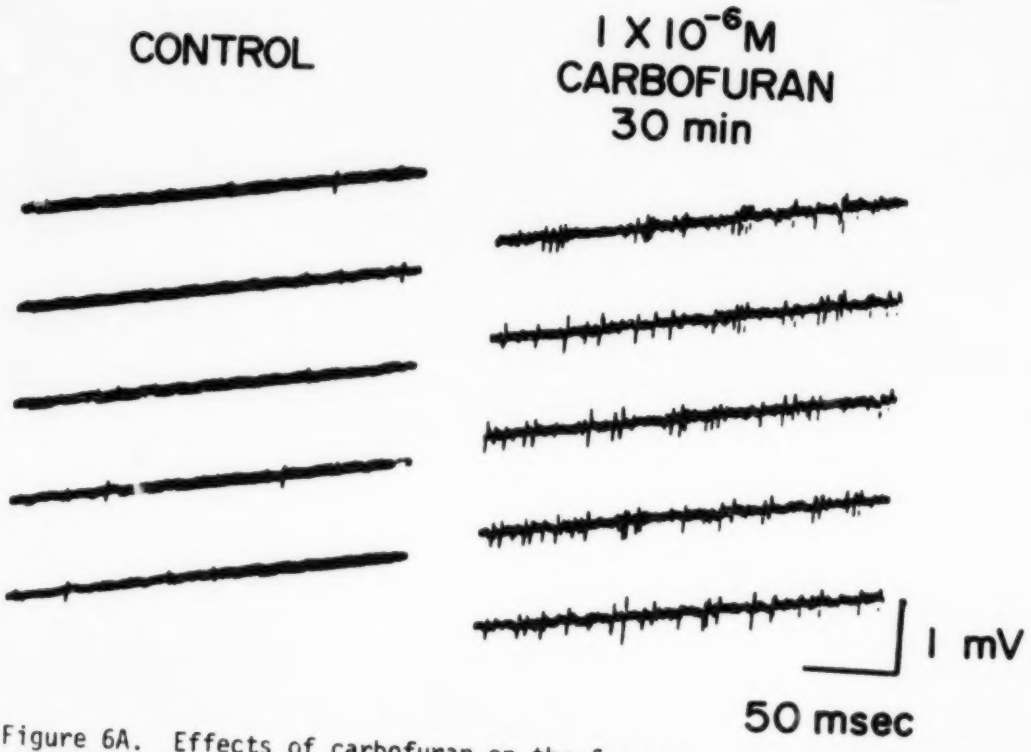

Figure 6A. Effects of carbofuran on the frequency of spontaneous discharges of the crayfish abdominal nerve cord. Each point represents a measurement during a period of 0.2 or 1 sec.

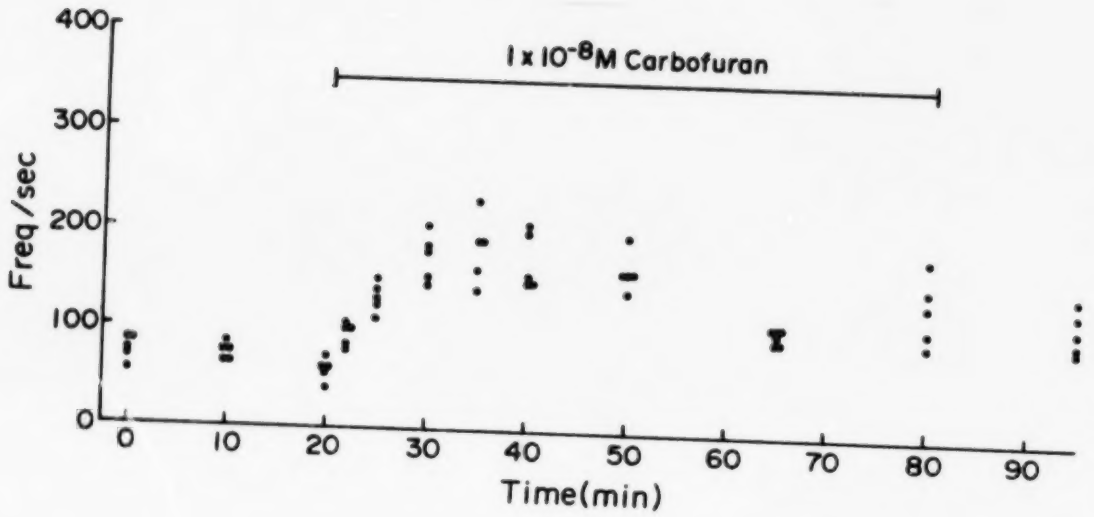

Figure 6B. Effects of carbofuran on the frequency of spontaneous discharges of the crayfish abdominal nerve cord. Each point represents a measurement during a period of 0.2 or 1 sec.

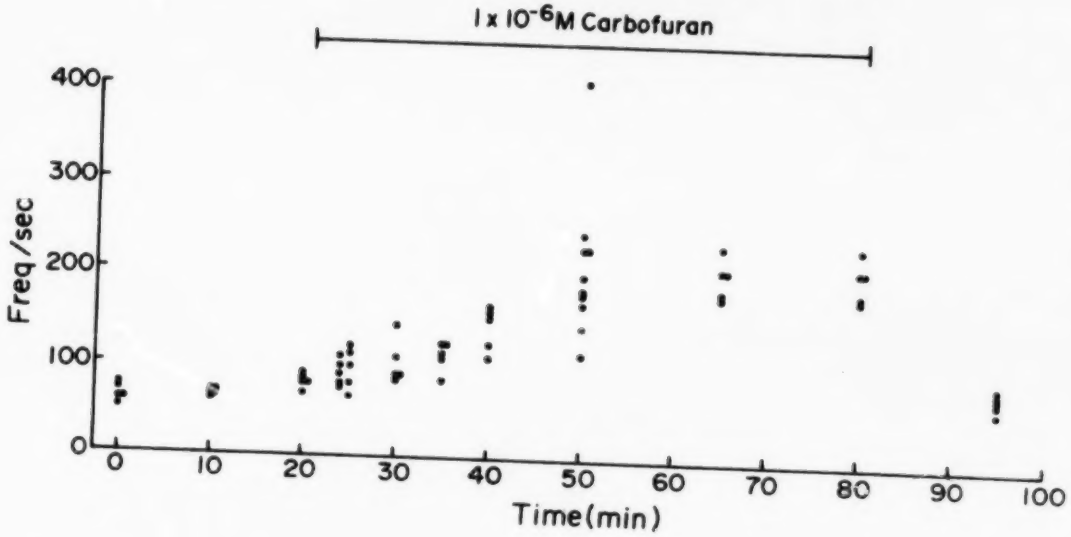

Figure 6C. Effects of carbofuran on the frequency of spontaneous discharges of the crayfish abdominal nerve cord. Each point represents a measurement during a period of 0.2 or 1 sec.

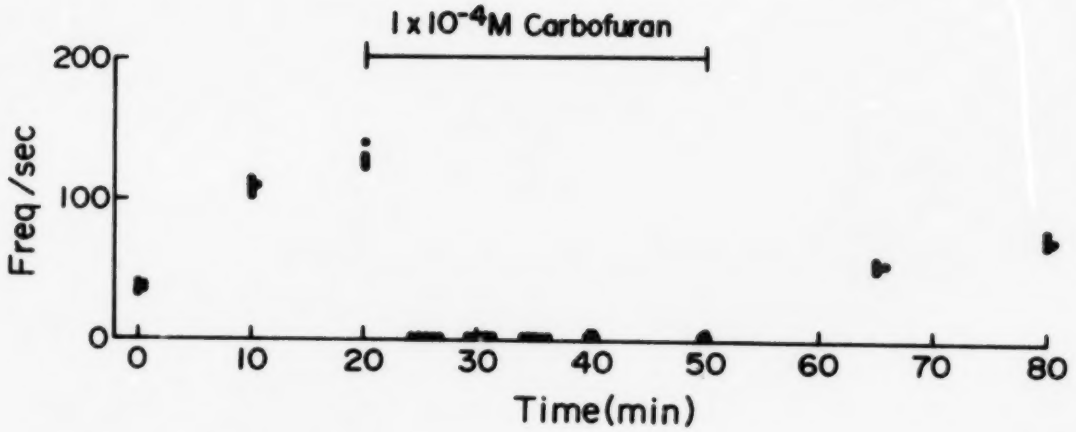

Figure 6D. Effects of carbofuran on the frequency of spontaneous discharges of the crayfish abdominal nerve cord. Each point represents a measurement during a period of 0.2 or 1 sec.

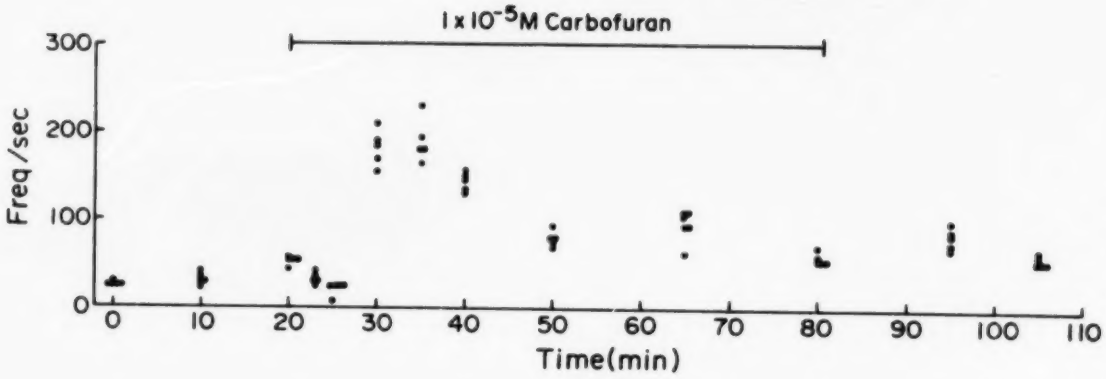

Figure 6E. Effects of carbofuran on the frequency of spontaneous discharges of the crayfish abdominal nerve cord. Each point represents a measurement during a period of 0.2 or 1 sec.

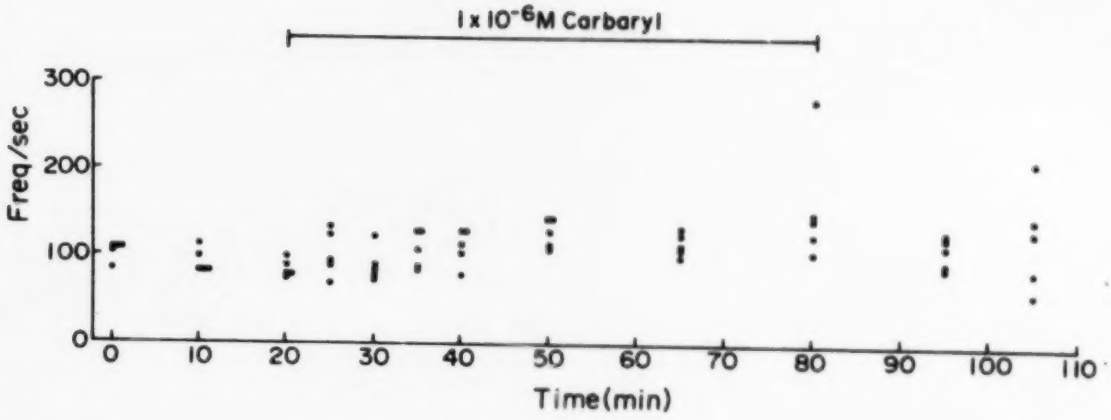

Figure 7A. Effects of carbaryl on the frequency of spontaneous discharges of the crayfish abdominal nerve cord. Each point represents a measurement during a period of 0.2 or 1 sec.

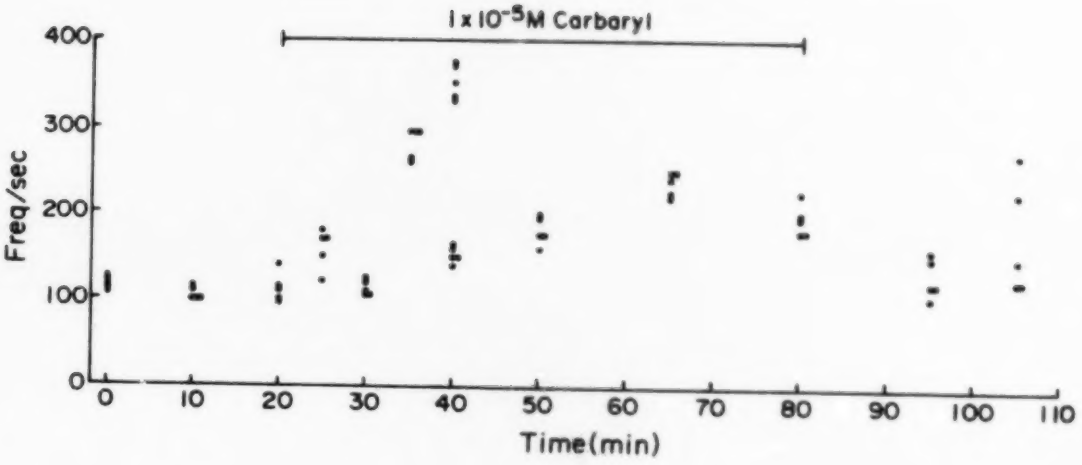

Figure 7B. Effects of carbaryl on the frequency of spontaneous discharges of the crayfish abdominal nerve cord. Each point represents a measurement during a period of 0.2 or 1 sec.

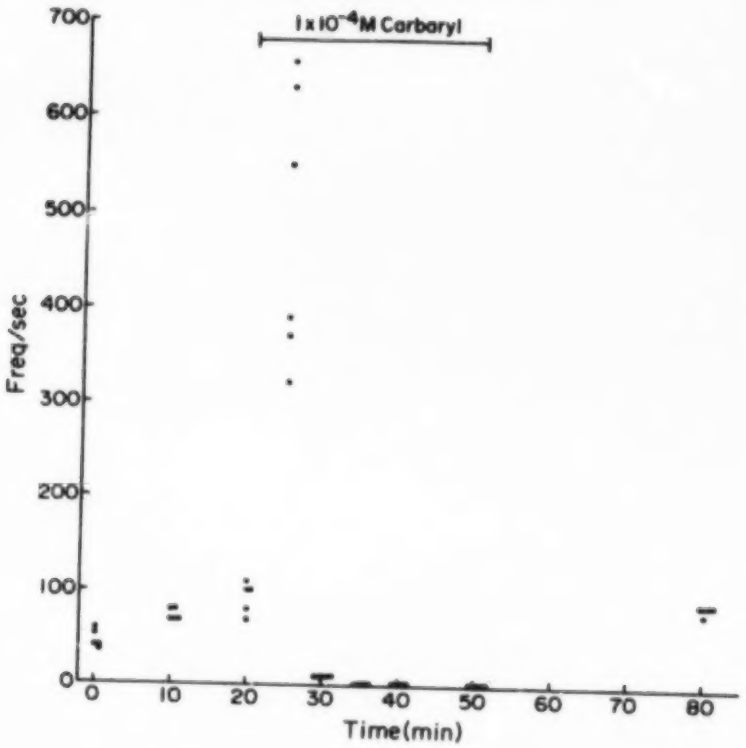

Figure 7C. Effects of carbaryl on the frequency of spontaneous discharges of the crayfish abdominal nerve cord. Each point represents a measurement during a period of 0.2 or 1 sec.

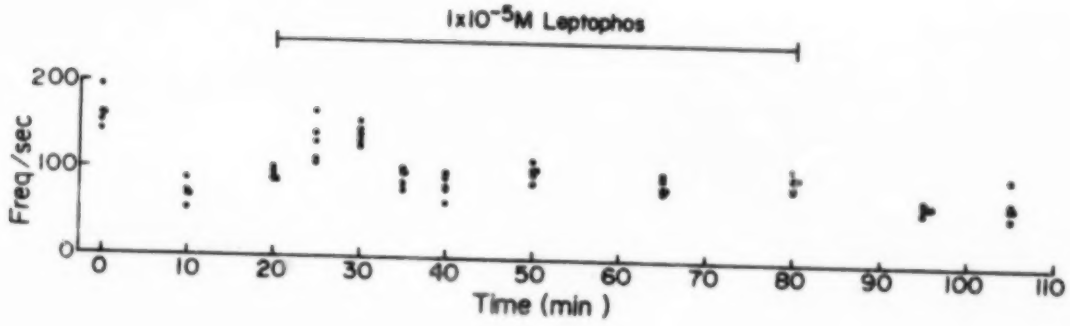

Figure 8A. Effects of leptophos on the frequency of spontaneous discharges of the crayfish abdominal nerve cord. Each point represents a measurement during a period of 0.2 or 1 sec.

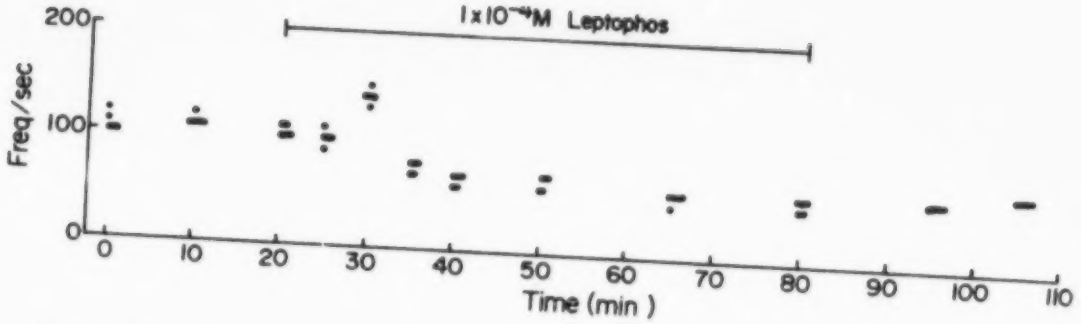

Figure 8B. Effects of leptophos on the frequency of spontaneous discharges of the crayfish abdominal nerve cord. Each point represents a measurement during a period of 0.2 or 1 sec.

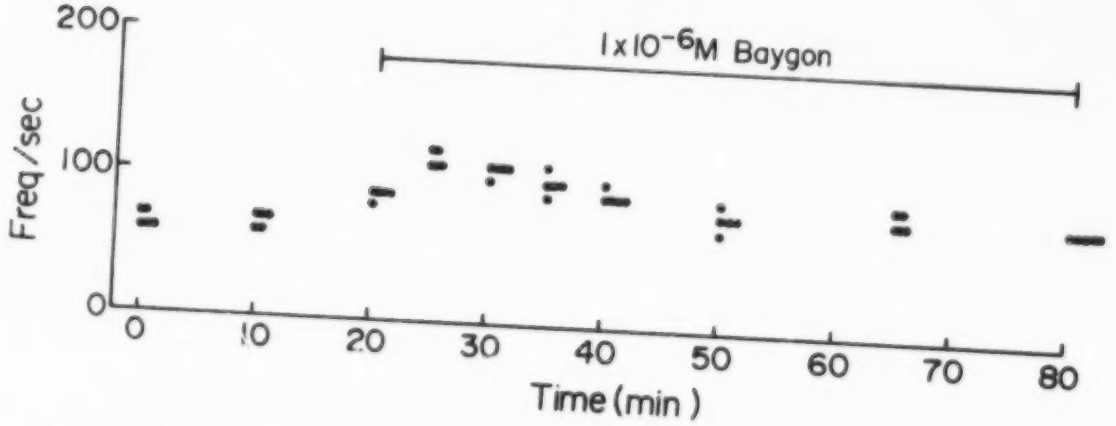

Figure 9A. Effects of Baygon (propoxur) on the frequency of spontaneous discharges of the crayfish abdominal nerve cord. Each point represents a measurement during a period of 0.2 or 1 sec.

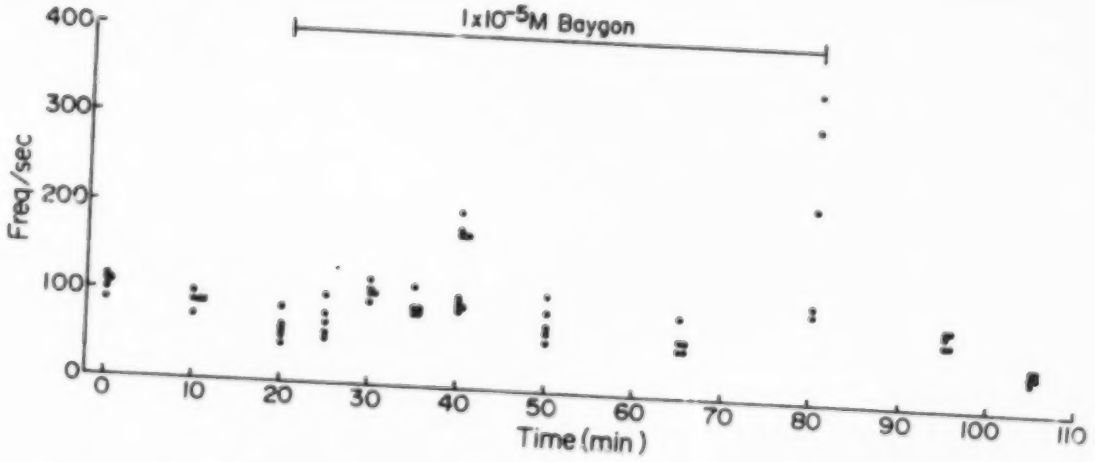

Figure 9B. Effects of Baygon (propoxur) on the frequency of spontaneous discharges of the crayfish abdominal nerve cord. Each point represents a measurement during a period of 0.2 or 1 sec.

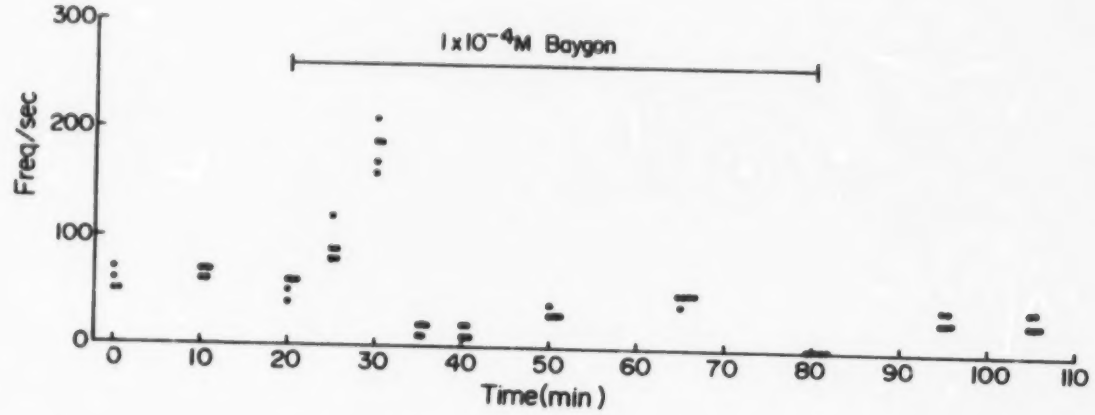

Figure 9C. Effects of Baygon (propoxur) on the frequency of spontaneous discharges of the crayfish abdominal nerve cord. Each point represents a measurement during a period of 0.2 or 1 sec.

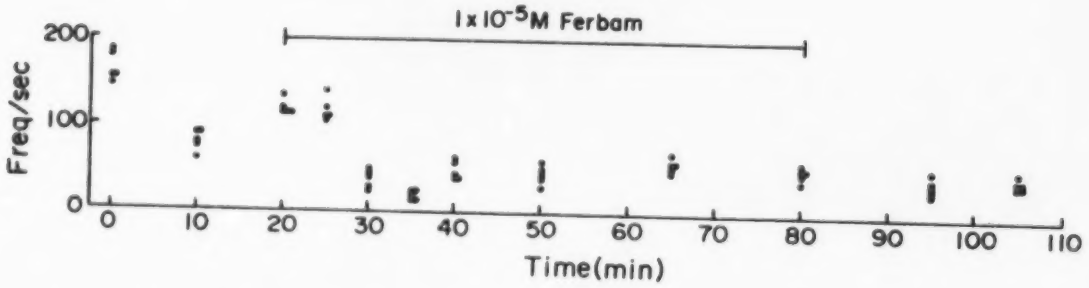

Figure 10A. Effects of ferbam on the frequency of spontaneous discharges of the crayfish abdominal nerve cord. Each point represents a measurement during a period of 0.2 or 1 sec.

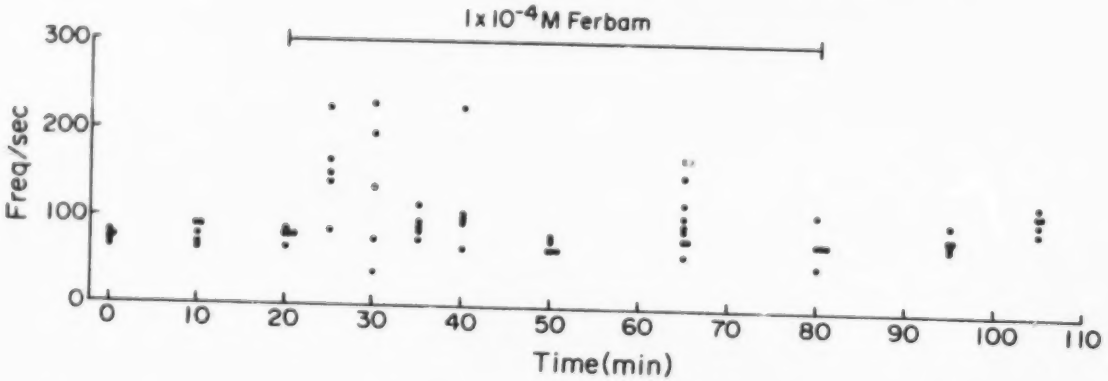

Figure 10B. Effects of ferbam on the frequency of spontaneous discharges of the crayfish abdominal nerve cord. Each point represents a measurement during a period of 0.2 or 1 sec.

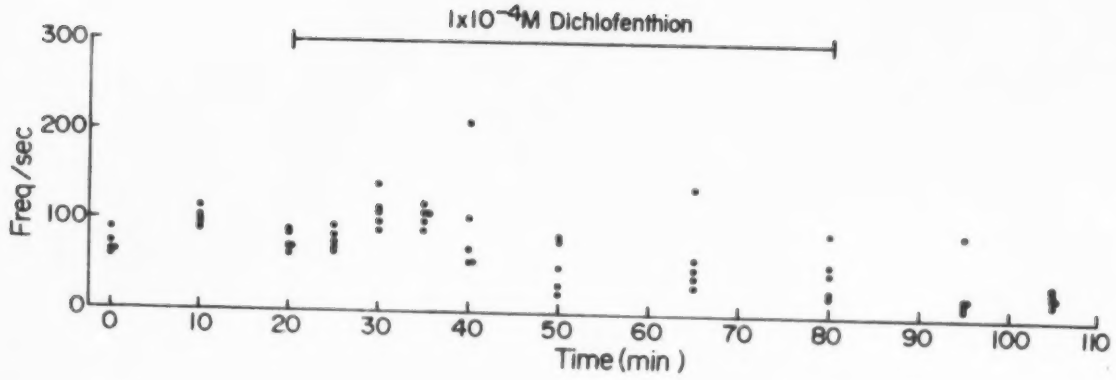

Figure 11. Effects of dichlofenthion on the frequency of spontaneous discharges of the crayfish abdominal nerve cord. Each point represents a measurement during a period of 0.2 or 1 sec.

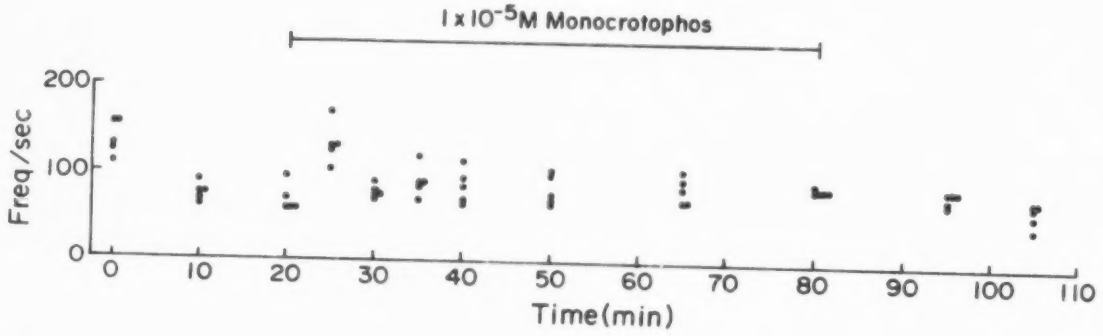

Figure 12A. Effects of monocrotophos on the frequency of spontaneous discharges of the crayfish abdominal nerve cord. Each point represents a measurement during a period of 0.2 or 1 sec.

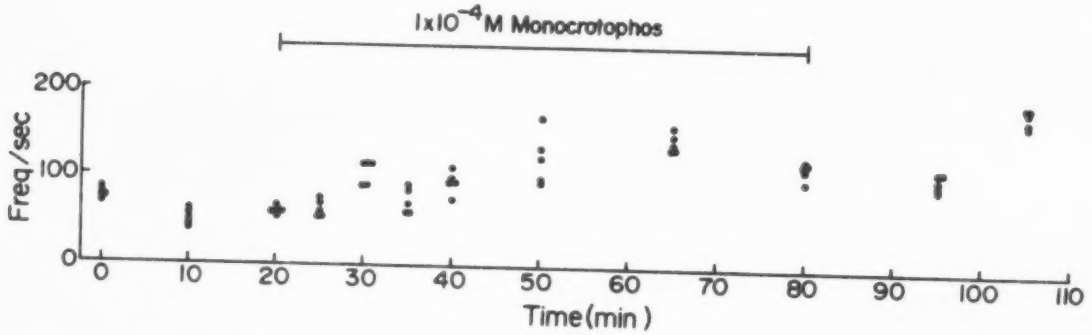

Figure 12B. Effects of monocrotophos on the frequency of spontaneous discharges of the crayfish abdominal nerve cord. Each point represents a measurement during a period of 0.2 or 1 sec.

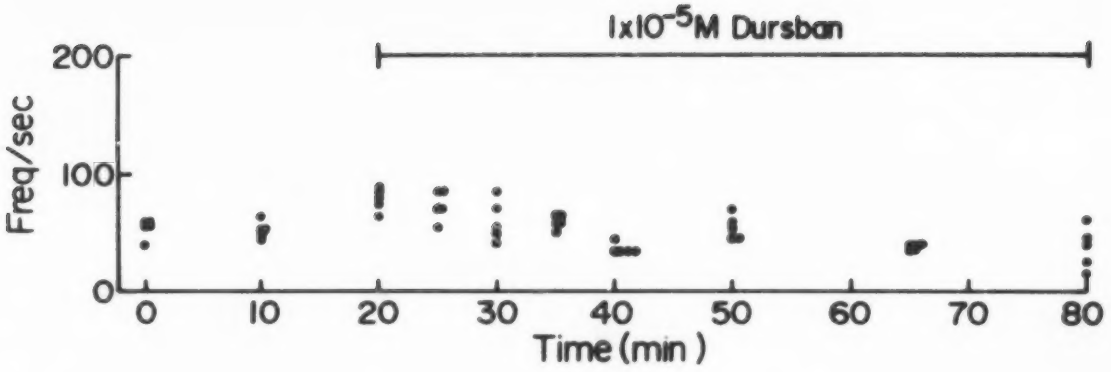

Figure 13A. Effects of dursban on the frequency of spontaneous discharges of the crayfish abdominal nerve cord. Each point represents a measurement during a period of 0.2 or 1 sec.

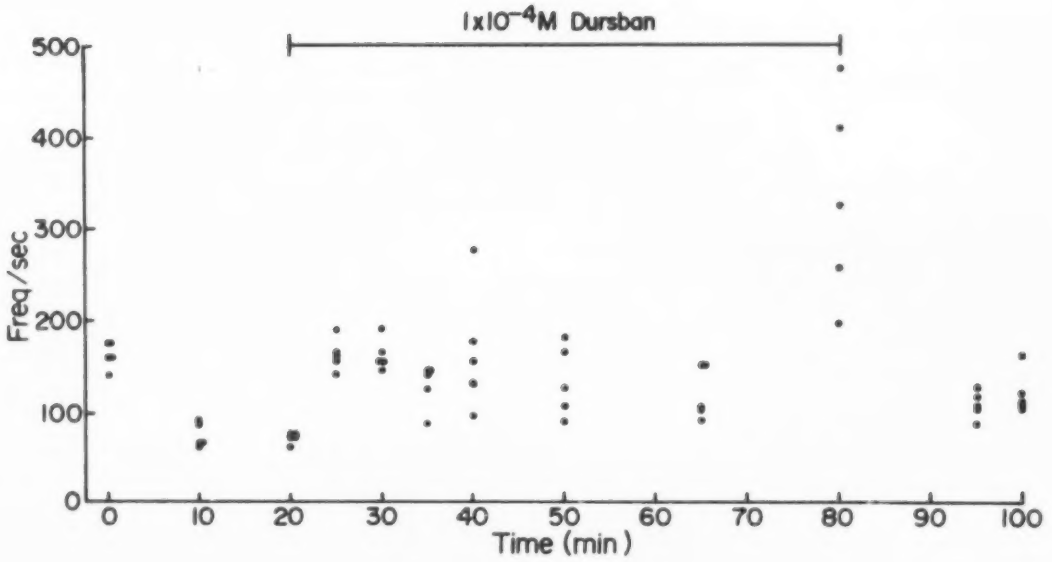

Figure 13B. Effects of dursban on the frequency of spontaneous discharges of the crayfish abdominal nerve cord. Each point represents a measurement during a period of 0.2 or 1 sec.

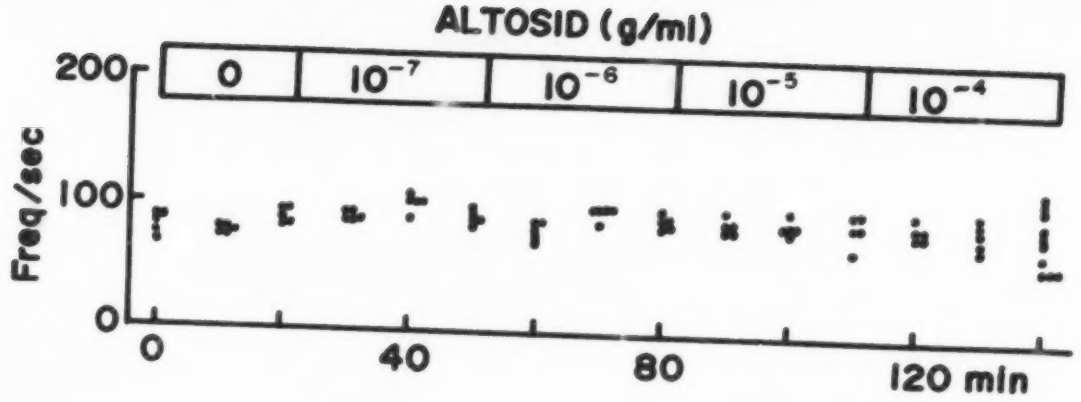

Figure 14. Effects of Altosid on the frequency of spontaneous discharges of the crayfish abdominal nerve cord. Each point represents a measurement during a period of 0.2 or 1 sec.

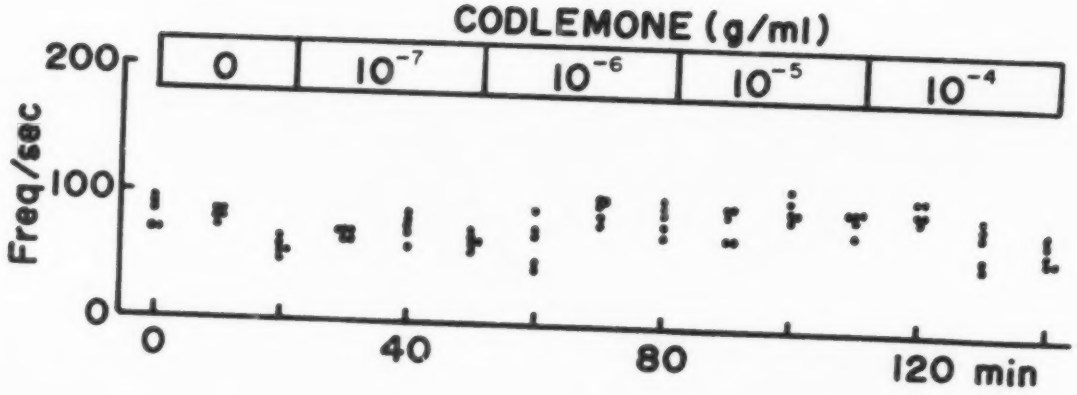

Figure 15. Effects of Codlemone on the frequency of spontaneous discharges of the crayfish abdominal nerve cord. Each point represents a measurement during a period of 0.2 or 1 sec.

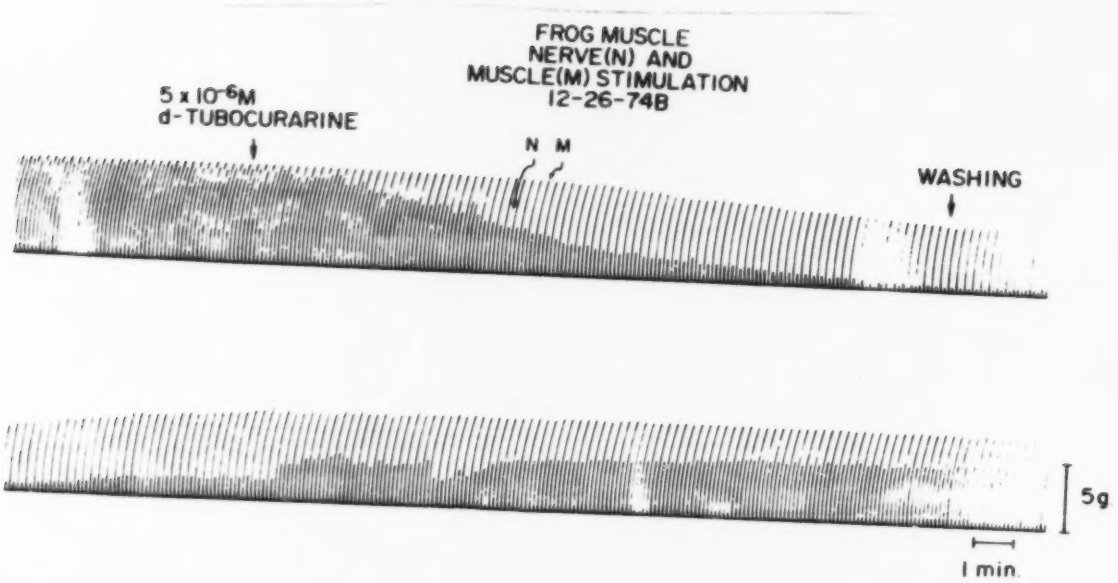

Figure 16. Effects of 5×10^{-6}M d-tubocurarine on the contractions of the frog sartorius muscle evoked by nerve(N) and muscle(M) stimulations.

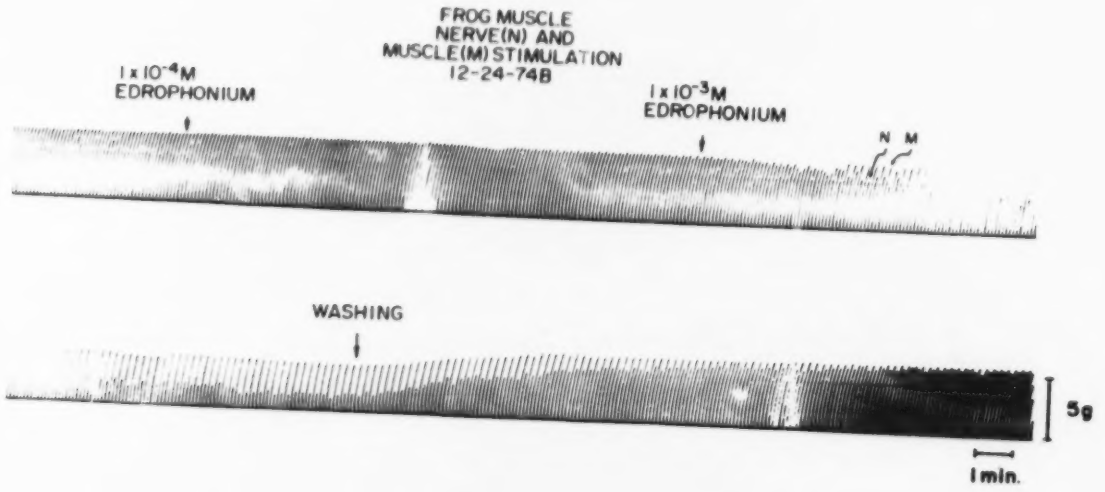

Figure 17. Effects of 1×10^{-3}M edrophonium on the contractions of the frog sartorius muscle evoked by nerve(N) and muscle(M) stimulations.

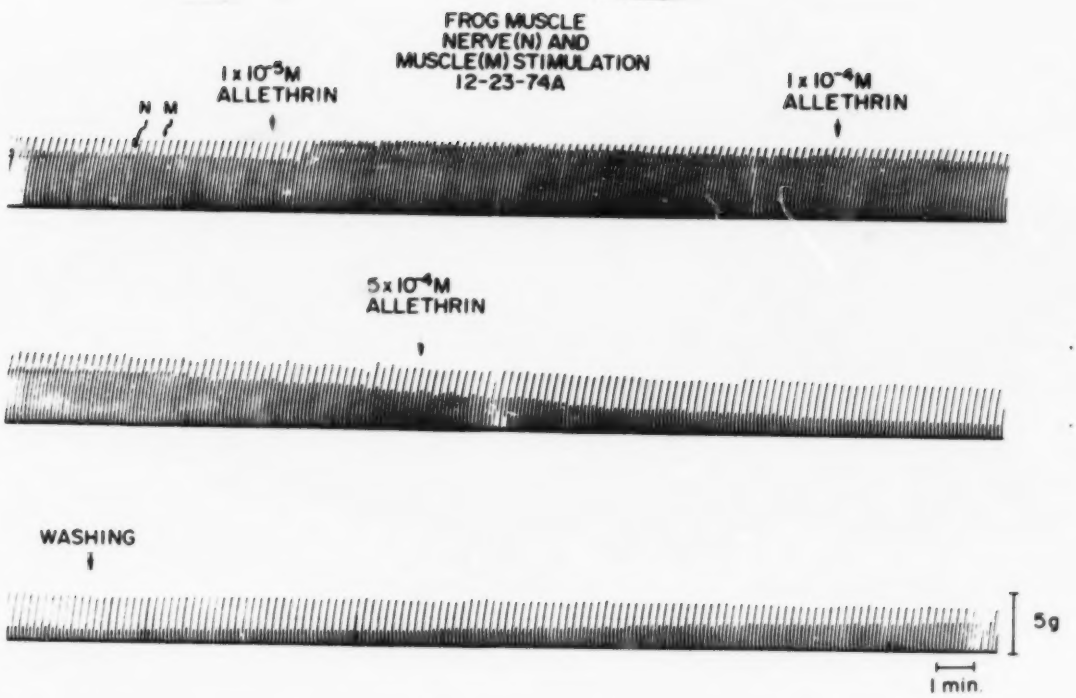

Figure 18. Effects of 1 x 10^{-5}M, 1 x 10^{-4}M, and 5 x 10^{-4}M allethrin on the contractions of the frog sartorius muscle evoked by nerve(N) and muscle(M) stimulations.

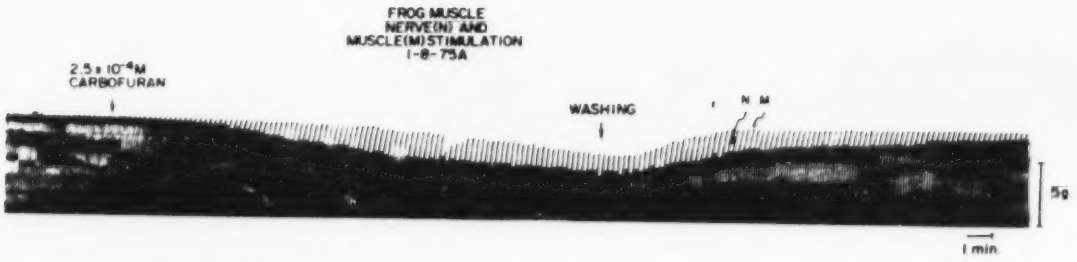

Figure 19. Effects of 2.5 x 10^{-4}M carbofuran on the contractions of the frog sartorius muscle evoked by nerve(N) and muscle(M) stimulations.

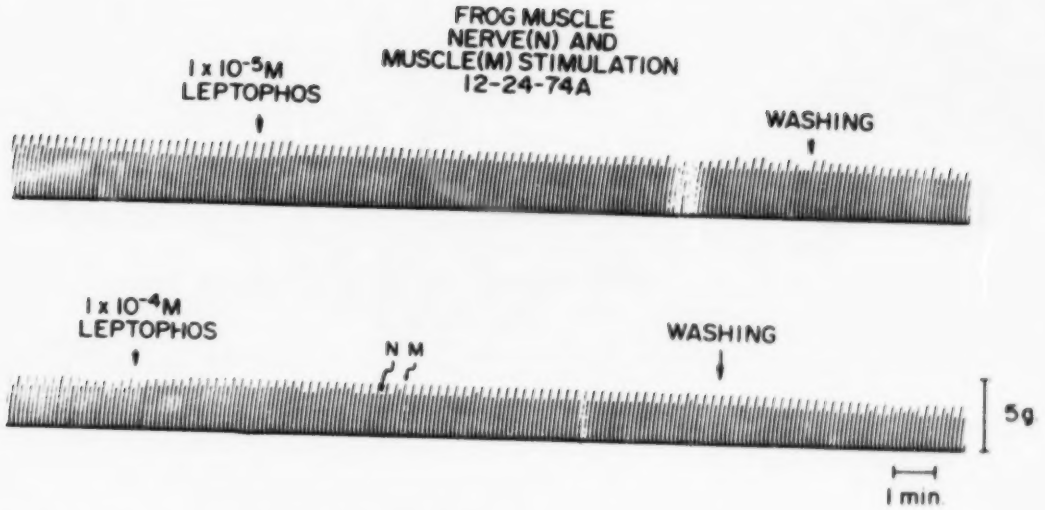

Figure 20. Effects of 1 x 10^{-4}M and 2.5 x 10^{-4}M leptophos on the contractions of the frog sartorius muscle evoked by nerve(N) and muscle(M) stimulations.

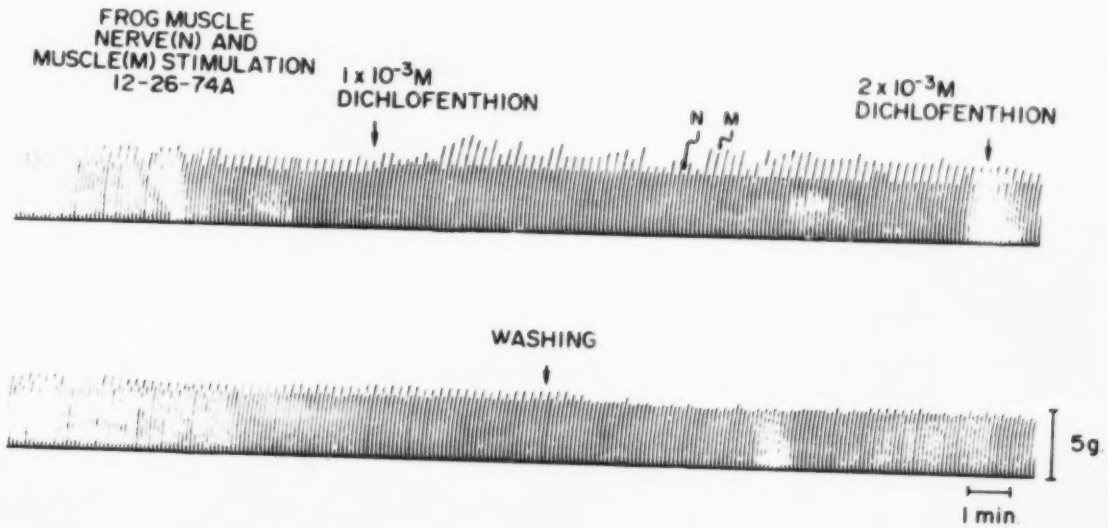

Figure 21. Effects of 1 x 10^{-3}M and 2 x 10^{-3}M dichlofenthion on the contractions of the frog sartorius muscle evoked by nerve(N) and muscle(M) stimulations.

Figure 22. Effects of 1 x 10^{-7}M allethrin on the contractions of the crayfish muscle evoked by nerve(N) and muscle(M) stimulations.

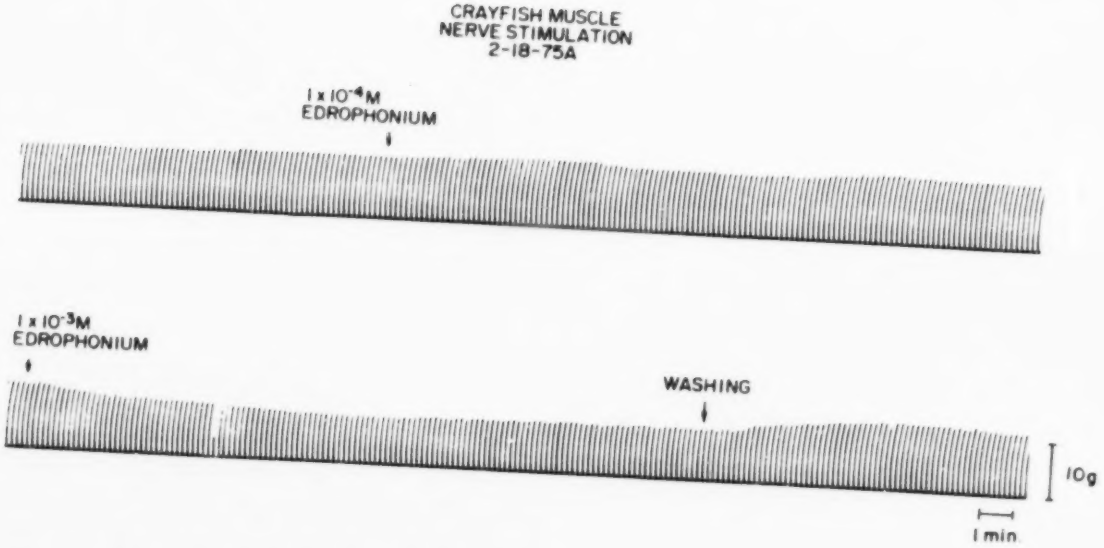

Figure 23. Effects of 1 x 10^{-4}M and 1 x 10^{-3}M edrophonium on the contractions of the crayfish muscle evoked by nerve stimulations.

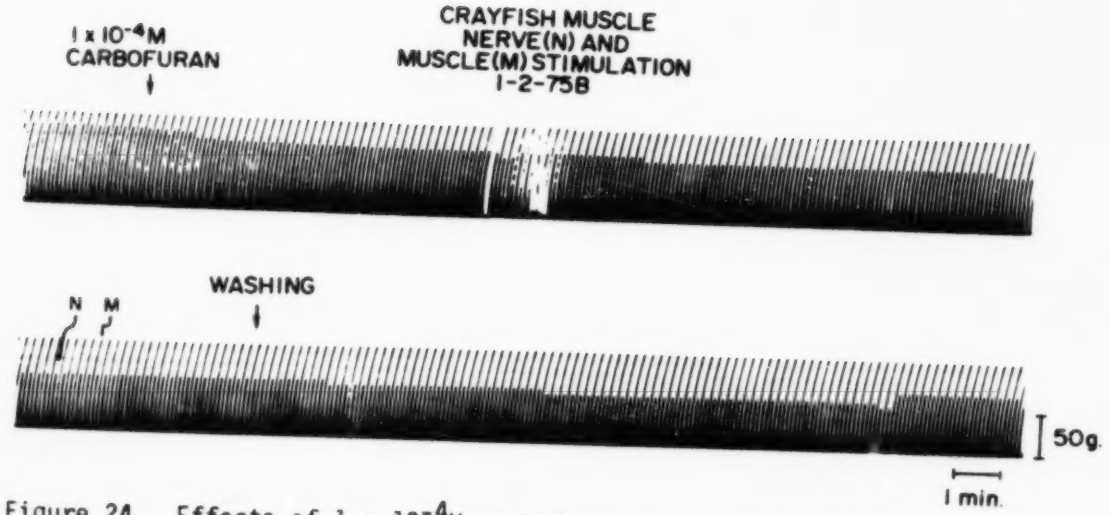

Figure 24. Effects of 1 x 10^{-4}M carbofuran on the contractions of the crayfish muscle evoked by nerve(N) and muscle(M) stimulations.

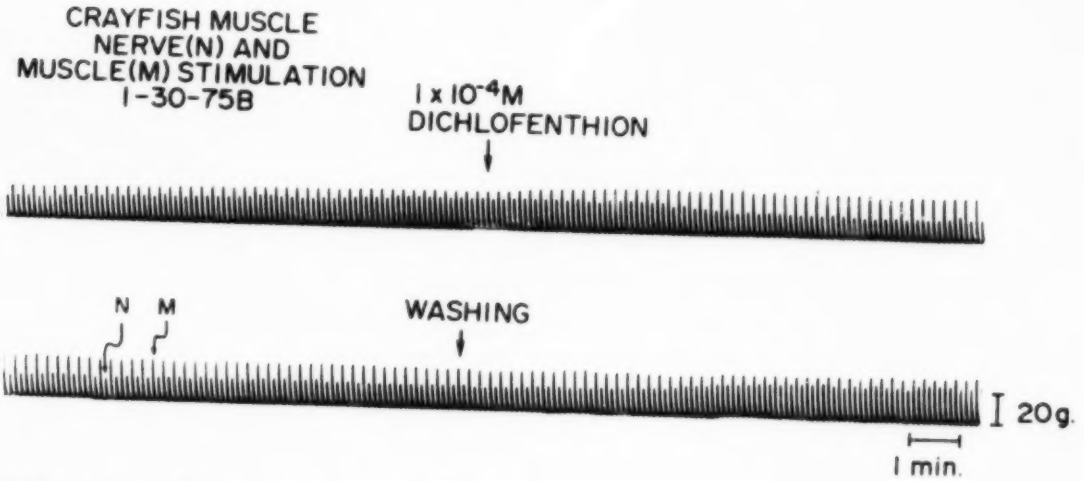

Figure 25. Effects of 1 x 10^{-4}M dichlofenthion on the contractions of the crayfish muscle evoked by nerve(N) and muscle(M) stimulations.

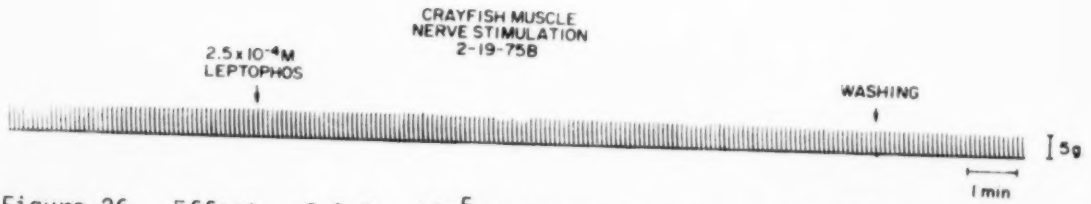

Figure 26. Effects of 2.5 x 10^{-5}M leptophos on the contractions of the crayfish muscle evoked by nerve stimulations.